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		DB=PGPB,USPT,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=ADJ	
☐	L3	L2 and (biotin or biotinylated or label$)	42
☐	L2	L1 and kinase	50
☐	L1	(FSBA or fluorosulfonylbenzoyl) and ATP	58

END OF SEARCH HISTORY

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L8 ANSWER 1 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2007:35391 HCAPLUS Full-text
DOCUMENT NUMBER: 146:243783
TITLE: Novel Rho kinase inhibitors with anti-inflammatory and vasodilatory activities
AUTHOR(S): Doe, Chris; Bentley, Ross; Behm, David J.; Lafferty, Robert; Stavenger, Robert; Jung, David; Bamford, Mark; Panchal, Terry; Grygielko, Eugene; Wright, Lois L.; Smith, Gary K.; Chen, Zunxuan; Webb, Christine; **Khandekar, Sanjay**; Yi, Tracey; Kirkpatrick, Robert; Dul, Edward; Jolivette, Larry; Marino, Joseph P., Jr.; Willette, Robert; Lee, Dennis; Hu, Erding
CORPORATE SOURCE: Department of Investigational Biology, Discovery Research, GlaxoSmithKline Pharmaceuticals Inc., King of Prussia, PA, USA
SOURCE: Journal of Pharmacology and Experimental Therapeutics (2007), 320(1), 89-98
CODEN: JPETAB; ISSN: 0022-3565
PUBLISHER: American Society for Pharmacology and Experimental Therapeutics
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Increased Rho kinase (ROCK) activity contributes to smooth muscle contraction and regulates blood pressure homeostasis. We hypothesized that potent and selective ROCK inhibitors with novel structural motifs would help elucidate the functional role of ROCK and further explore the therapeutic potential of ROCK inhibition for hypertension. In this article, we characterized two aminofurazan-based inhibitors, GSK269962A [N-(3-{[2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-1H-imidazo[4,5-c]pyridin- 6-yl]oxy}phenyl)-4-{[2-(4-morpholinyl)ethyl]-oxy}benzamide] and SB-7720770-B [4-(7-{[(3S)-3-amino-1-pyrrolidinyl]carbonyl}-1-ethyl-1H- imidazo[4,5-c]pyridin-2-yl)-1,2,5-oxadiazol-3-amine], as members of a novel class of compds. that potently inhibit ROCK **enzymic** activity. GSK269962A and SB-772077-B have IC50 values of 1.6 and 5.6 nM toward recombinant human ROCK1, resp. GSK269962A also exhibited more than 30-fold selectivity against a panel of serine/threonine kinases. In lipopolysaccharide-stimulated monocytes, these inhibitors blocked the generation of inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α. Furthermore, both SB-772077-B and GSK269962A induced vasorelaxation in preconstricted rat aorta with an IC50 of 39 and 35 nM, resp. Oral administration of either GSK269962A or SB-772077-B produced a profound dose-dependent reduction of systemic blood pressure in spontaneously hypertensive rats. At doses of 1, 3, and 30 mg/kg, both compds. induced a reduction in blood pressure of approx. 10, 20, and 50 mm Hg. In addition, administration of SB-772077-B also dramatically lowered blood pressure in DOCA salt-induced hypertensive rats. SB-772077-B and GSK269962A represent a novel class of ROCK inhibitors that have profound effects in the vasculature and may enable us to further evaluate the potential beneficial effects of ROCK inhibition in animal models of cardiovascular as well as other chronic diseases.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2006:1324378 HCAPLUS Full-text
DOCUMENT NUMBER: 146:220117
TITLE: Discovery of Aminofurazan-azabenzimidazoles as Inhibitors of Rho-Kinase with High Kinase Selectivity

MJR

and Antihypertensive Activity

AUTHOR(S): Stavenger, Robert A.; Cui, Haifeng; Dowdell, Sarah E.; Franz, Robert G.; Gaitanopoulos, Dimitri E.; Goodman, Krista B.; Hilfiker, Mark A.; Ivy, Robert L.; Leber, Jack D.; Marino, Joseph P., Jr.; Oh, Hye-Ja; Viet, Andrew Q.; Xu, Weiwei; Ye, Guosen; Zhang, Daohua; Zhao, Yongdong; Jolivette, Larry J.; Head, Martha S.; Semus, Simon F.; Elkins, Patricia A.; Kirkpatrick, Robert B.; Dul, Edward; **Khandekar, Sanjay S.** ; Yi, Tracey; Jung, David K.; Wright, Lois L.; Smith, Gary K.; Behm, David J.; Doe, Christopher P.; Bentley, Ross; Chen, Zunxuan X.; Hu, Erding; Lee, Dennis

CORPORATE SOURCE: Department of Medicinal Chemistry, GlaxoSmithKline, King of Prussia, PA, 19406, USA

SOURCE: Journal of Medicinal Chemistry (2007), 50(1), 2-5
CODEN: JMCMAR; ISSN: 0022-2623

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

OTHER SOURCE(S): CASREACT 146:220117

AB The discovery, proposed binding mode, and optimization of a novel class of Rho-kinase inhibitors are presented. Appropriate substitution on the 6-position of the azabenzimidazole core provided subnanomolar **enzyme** potency in vitro while dramatically improving selectivity over a panel of other kinases. Pharmacokinetic data was obtained for the most potent and selective examples and one (6n) has been shown to lower blood pressure in a rat model of hypertension.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2004:799559 HCAPLUS Full-text

DOCUMENT NUMBER: 141:291247

TITLE: Methods for identifying **enzyme** inhibitors: affinity labeling of the ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine

INVENTOR(S): **Bramson, Harold Neal; Glover, George I.**; Khandekar, Sanjav; **Ratcliffe, Steven John**

PATENT ASSIGNEE(S): Smithkline Beecham Corporation, USA

SOURCE: PCT Int. Appl., 26 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004083175	A2	20040930	WO 2004-US8043	20040317
WO 2004083175	A3	20050203		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE,

ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG.

EP 1604021	A2	20051214	EP 2004-757519	20040317
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK				
JP 2006520600	T	20060914	JP 2006-507244	20040317
US 2006234327	A1	20061019	US 2005-549390	20050914
PRIORITY APPLN. INFO.:			US 2003-455374P	P 20030317
			US 2003-487983P	P 20030717
			WO 2004-US8043	W 20040317

AB The present invention provides methods for identifying compds. that inhibit kinases. In addition, methods for profiling **protein kinases** are also provided. In addition, methods for determining the mode of action of kinase inhibitors are also provided. P-Fluorosulfonylbenzoyl 5'-adenosine (FSBA) is an ATP-affinity reagent that is effective in covalent modification of nucleotide-binding sites in a variety of **protein kinases**. For initial FSBA labeling studies, recombinantly expressed and purified kinase domain of the transforming growth factor (TGF)-β type I receptor (activin receptor-like kinase, ALK5) was used. Autoradiog. results indicated that FSBA labeled unheated ALK5 and CDK2 but did not label heat-denatured ALK5. Similar results were obtained for a panel of other kinases. The results indicate that FSBA and ATP compete for the same binding pocket in ALK5. Together the heat denaturation and ATP protection results indicated that FSBA has potential to be an activity-based probe for kinase profiling studies. Time-dependent reactions were performed to assess FSBA modification of kinases by LC/MS. ATP protected covalent modification of both ALK5 and ALK4 by FSBA in a concentration-dependent manner; most of the covalent modification was inhibited in the presence of 0.5 mM ATP. Likewise ATP protected labeling of CDK2 with FSBA in a concentration-dependent manner, although 5 mM ATP was necessary to block FSBA binding. FSBA binding to all **enzymes** tested was essentially blocked by the addition of 10 μM staurosporine. Synthesis of biotinylated FSBA [5'-p- fluorosulfonylbenzoyl-(2' or 3')-biotinyladenosine] is described. The results indicate that biotinylated FSBA modifies **protein kinase** as effectively as non-biotinylated FSBA. Similarly, these results indicate that LC/MS may be used to detect biotin-FSBA association with a kinase. One embodiment of the present invention is to provide a method for identifying a compound that inhibits an **enzyme** having an ATP binding site comprising contacting a, composition of the **enzyme**, an analyte capable of binding to the ATP binding site of the **enzyme** , and a test compound, and detecting whether the test compound inhibits the analyte from binding the ATP binding site of the **enzyme**.

L8 ANSWER 4 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER:	1992:5122 HCAPLUS Full-text
DOCUMENT NUMBER:	116:5122
TITLE:	Synthetic fragments of the CD4 receptor cytoplasmic domain and large polycations alter the activities of the pp56lck tyrosine **protein kinase**
AUTHOR(S):	**Bramson, H. Neal**; Casnellie, John E.; Nachod, Holly; Regan, Lisa M.; Sommers, Christine
CORPORATE SOURCE:	Med. Cent., Univ. Rochester, Rochester, NY, 14642, USA
SOURCE:	Journal of Biological Chemistry (1991), 266(24), 16219-25 CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB In CD4+ T cells, the src-like tyrosine kinase pp56lck is associated with the CD4 receptor and crosslinking of CD4 results in the activation of this **enzyme**.

The mechanism responsible for this activation is not known, although there is evidence that the activities of the src family of **enzymes** are regulated by tyrosine phosphorylation. Here is reported that pp56lck-catalyzed angiotensin II phosphorylations are activated 20-fold in vitro by synthetic peptides reproducing portions of the murine CD4 cytoplasmic domain. This activation has a dissociation constant of about 2 μM. The pp56lck-catalyzed phosphorylation of other peptide substrates are effected less and in one case not at all by the peptide modulators, indicating that these CD4 sequences alter the substrate specificity of pp56lck. In contrast, peptides reproducing sequences from the CD8 receptor have a charge and size similar to the CD4 peptides, yet are vastly less effective at modulating pp56lck activities. High ionic strengths inhibit the CD4 peptide-induced modulation of pp56lck phosphotransferase activities, suggesting that charge-charge interactions are important for this process. In addition, the modulation of pp56lck activities by peptides reproducing the CD4 cytoplasmic domain are reproduced by polycations significantly larger than the CD4 cytoplasmic domain but not by those of similar size. The modulations both by CD4 peptides and the polycations do not depend on **enzyme** tyrosine phosphorylations.

L8 ANSWER 5 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1987:454699 HCAPLUS Full-text
DOCUMENT NUMBER: 107:54699
TITLE: Conformation of Leu-Arg-Arg-Ala-Ser-Leu-Gly bound in the active site of adenosine cyclic 3',5'-phosphate dependent **protein kinase**
AUTHOR(S): **Bramson, H. Neal**; Thomas, Nancy E.; Miller, W. Todd; Fry, David C.; Mildvan, Albert S.; Kaiser, E. T.
CORPORATE SOURCE: Lab. Bioorg. Chem. Biochem., Rockefeller Univ., New York, NY, 10021, USA
SOURCE: Biochemistry (1987), 26(14), 4466-70
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Studies utilizing NMR spectroscopy have shown that cAMP-dependent **protein kinase** (A-kinase) probably binds Leu-Arg-Arg-Ala-Ser-Leu-Gly (peptide 1) in 1 of 2 extended coil conformations (A or B). The relative reactivities of a series of N-methylated peptides based on the structure of peptide 1 might, therefore, be related to how well each can assume the A or B conformation. From ests. of the magnitude of steric interactions that would be induced by N-methylation of an amide in peptide 1 that is locked in either conformation, the ability of each peptide to form that conformation was predicted. The ability of A-kinase to catalyze phosphorylation of the N-methylated peptides correlated well with the ability of each peptide to form conformation A, but not conformation B. In accord with these findings, the reactivity of an unreactive N-methylated peptide was partially restored by a second change, which allowed the peptide to assume conformation A. These results suggest that when bound in the **enzymic** active site, peptide 1 has a conformation that resembles structure A much more closely than structure B.

L8 ANSWER 6 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1987:454698 HCAPLUS Full-text
DOCUMENT NUMBER: 107:54698
TITLE: Distinguishing among **protein kinases** by substrate specificities
AUTHOR(S): Thomas, Nancy E.; **Bramson, H. Neal**; Nairn, Angus C.; Greengard, Paul; Fry, David C.; Mildvan, Albert S.; Kaiser, E. T.

CORPORATE SOURCE: Lab. Bioorg. Chem. Biochem., Rockefeller Univ., New York, NY, 10021, USA
SOURCE: Biochemistry (1987), 26(14), 4471-4
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Conformationally restricted N-methylated peptides were used to probe the active site of cGMP-dependent **protein kinase** (G-kinase), which is homologous in sequence and has substrate specificities similar to those of (cAMP-dependent) A-kinase. Although this **enzyme** appears to bind the peptides in a conformation resembling that of conformation A, it is more able to accommodate backbone methylation than is A-kinase. A peptide substrate ≥700-fold more selective for G-kinase than for A-kinase was found. Backbone methylation may, therefore, represent a way of making peptide substrates and inhibitors selective for a particular kinase.

L8 ANSWER 7 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1987:454697 HCAPLUS Full-text
DOCUMENT NUMBER: 107:54697
TITLE: Role of **enzyme**-peptide substrate backbone hydrogen bonding in determining **protein kinase** substrate specificities
AUTHOR(S): Thomas, Nancy E.; **Bramson, H. Neal**; Miller, W. Todd; Kaiser, E. T.
CORPORATE SOURCE: Lab. Bioorg. Chem. Biochem., Rockefeller Univ., New York, NY, 10021, USA
SOURCE: Biochemistry (1987), 26(14), 4461-6
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB As part of a search for peptides that have specificity for selected **protein kinases**, the possibility that cAMP-dependent **protein kinase** (A-kinase) recognizes the H-bonding potential of its peptide substrates was investigated. A-kinase catalyzes the phosphorylation of 5 Nα-methylated and 4 depsipeptide derivs. of Leu-Arg-Arg-Ala-Ser-Leu-Gly (peptide 1) at rates that differ by ≥7 orders of magnitude. These peptide 1 analogs each lack the ability to donate a H bond at selected positions in the peptide chain. If a particular amide H of a peptide amide is involved in H bonding, which is important for **enzyme** recognition, the prediction is that peptides which contain an ester or a N-methylated bond at that position in peptide 1 will be comparatively poor substrates. In contrast, if a depsipeptide has a reactivity comparable to that of peptide 1 but the analogous N-methylated peptide has a poor reactivity with A-kinase, the result might indicate that the N-Me group causes unfavorable steric effects. The depsipeptide that lacks a leucine-6 amide proton is a good substrate for A-kinase, but the corresponding N-methylated peptide is phosphorylated far less efficiently. This result and others presented in this paper suggest that although **enzyme**-substrate H bonding may play some role in A-kinase catalysis of phosphoryl group transfer, other explanations are necessary to account for the relative reactivities of Nα-methylated and depsi-containing peptide 1 analogs. Alternate explanations that cannot be eliminated from the data presented here include the presence of disruptive peptide-**enzyme** steric interactions or intrapeptide steric interactions that might prevent a peptide 1 analog from assuming a conformation recognizable by A-kinase.

L8 ANSWER 8 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1985:609679 HCAPLUS Full-text

DOCUMENT NUMBER: 103:209679
TITLE: The use of N-methylated peptides and depsipeptides to probe the binding of heptapeptide substrates to cAMP-dependent **protein kinase**
AUTHOR(S): **Bramson, H. Neal**; Thomas, Nancy E.; Kaiser, Emil Thomas
CORPORATE SOURCE: Lab. Bioorg. Chem. Biochem., Rockefeller Univ., New York, NY, 10021, USA
SOURCE: Journal of Biological Chemistry (1985), 260(29), 15452-7
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Analogs of Leu-Arg-Arg-Ala-Ser-Leu-Gly (I) that contain depsi linkages replacing selected amide bonds are good substrates for cAMP-dependent **protein kinase** (II). Therefore, with the possible exception of the serine amide proton, no I amide H atoms are involved in peptide-peptide or peptide-**enzyme** H-bonding crucial to defining the high substrate activity of this peptide. It is thus unlikely that I is bound by I while in an α-helical or a β-turn structure. Three peptides were very poor substrates for II: namely, those containing N-Me amino acids in place of serine-6 or leucine-6 and a peptide containing proline in place of leucine-6. These peptides are poor substrates for the **enzyme** possibly because they are unable to adopt a conformation necessary for catalysis of phosphoryl group transfer to occur or due to steric effects in the **enzyme** active site.

L8 ANSWER 9 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1984:81929 HCAPLUS Full-text
DOCUMENT NUMBER: 100:81929
TITLE: Use of NMR and EPR to study cAMP-dependent **protein kinase**
AUTHOR(S): Mildvan, Albert S.; Rosevear, Paul R.; Granot, Joseph; O'Brian, Catherine A.; **Bramson, H. Neil**; Kaiser, E. T.
CORPORATE SOURCE: Sch. Med., Johns Hopkins Univ., Baltimore, MD, 21205, USA
SOURCE: Methods in Enzymology (1983), 99(Horm. Action, Part F), 93-119
CODEN: MENZAU; ISSN: 0076-6879
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The principles and use of EPR and NMR methods to study cAMP-dependent **protein kinase** are discussed with reference to determining the conformation and arrangement of the substrates, cations, and cAMP in the **enzyme** binding sites. The interactions of these factors with the catalytic and regulatory subunits, as well as the interactions of the subunits themselves are topics included.

L8 ANSWER 10 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1983:418603 HCAPLUS Full-text
DOCUMENT NUMBER: 99:18603
TITLE: Nuclear Overhauser effect studies of the conformations of tetraamminecobalt(III)-ATP free and bound to bovine heart **protein kinase**
AUTHOR(S): Rosevear, Paul R.; **Bramson, H. Neal**; O'Brian, Catherine; Kaiser, E. T.; Mildvan, Albert S.
CORPORATE SOURCE: Sch. Med., Johns Hopkins Univ., Baltimore, MD, 21205, USA
SOURCE: Biochemistry (1983), 22(14), 3439-47

CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Nuclear Overhauser effects (NOE) and selective spin-lattice relaxation (T1) values were used to determine interproton distances on Co(NH3)4ATP, free in solution and bound to the catalytic subunit of **protein kinase**. The distances in free Co(NH3)4ATP could not be fit by a single conformation, and were therefore assumed to result from the averaging of the principal conformations that have been detected by x-ray anal., and theor. calculated to be at or near energy min. Fitting of the interproton distances required the averaging of ≥3 of these nucleotide conformations. According to these calcns., 2 anti conformers with glycosidic torsional angles centering at 15° and 55° with extreme C3'-endo and O1'-endo ribose puckers, resp., contributed .apprx.86% to the average structure. A syn conformer with a glycosidic torsional angle centering at 217° and a C2'-endo ribose pucker contributed .apprx.14% to the average structure. The NOE studies thus established the averaging of several conformations of free Co(NH3)4ATP. Previous paramagnetic probe-T1 studies of the binary complex of Mn2+ and Co(NH3)4ATP when interpreted as a root-mean-6th average of distances in these 3 conformers, yielded, within exptl. error, the measured Mn2+-proton distances. In contrast, the interproton distances on **enzyme** -bound Co(NH3)4ATP were fit by a single nucleotide conformation with a high anti glycosidic torsional angle (χ = 78°) and an O1'-endo ribose pucker or a mixture of ribose puckers. This conformation of Co(CH3)4ATP, which is unaltered by saturating the inhibitory site of the **enzyme** with Mg2+, is indistinguishable from 1 of 2 alternative conformations, previously determined by distances from Mn2+ at the inhibitory site of the **enzyme** to the protons and P nuclei of bound Co(NH3)4ATP. The consistency of the conformations of **enzyme** -bound Co(NH3)4ATP found by 2 independent methods with differing reference points and observation frequencies indicates a unique conformation of the bound nucleotide. As **protein kinase** loses activity with time, the interproton NOE's change in a manner indicating that Co(NH3)4ATP remains bound to the **enzyme**. However, the protein structure at the nucleotide binding site alters, allowing the glycosidic conformational angle of Co(NH3)4ATP to relax to a lower anti value.

L8 ANSWER 11 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1982:595115 HCAPLUS Full-text
DOCUMENT NUMBER: 97:195115
TITLE: Modification of the catalytic subunit of bovine heart cAMP-dependent **protein kinase** with affinity labels related to peptide substrates
AUTHOR(S): **Bramson, H. Neal**; Thomas, Nancy; Matsueda, Rei; Nelson, Norman C.; Taylor, Susan S.; Kaiser, Emil Thomas
CORPORATE SOURCE: Searle Chem. Lab., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Journal of Biological Chemistry (1982), 257(18), 10575-81
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The modification and concomitant inactivation of the catalytic subunit of bovine heart cAMP-dependent **protein kinase** with affinity analogs of peptide substrates potentially capable of undergoing disulfide interchange with **enzyme**-bound SH groups were used to probe the active site associated with peptide binding. The regeneration of catalytic activity on treatment of the modified **enzymes** with dithiothreitol and the observation that prior reaction with 5,5'-dithiobis(2-nitrobenzoic acid) blocks the modification of the kinase by these reagents were consistent with the proposal that only SH residues were

reacting. The affinity analog, Leu-Arg-Arg-Ala-Cys(3-nitro-2-pyridinesulfenyl)-Leu-Gly (I), and the closely related peptide, AcLeu-Arg-Arg-Ala-Cys(3-nitro-2-pyridinesulfenyl)-Leu-Gly-OEt (II), reacted with a single SH group, as shown by the stoichiometry of the release of the 3-nitro-2-pyridinesulfenyl group and the amount of label incorporated in the **enzyme** when the radioactively labeled peptide analog of II was employed as the modifying agent. The kinetics of the reaction of I with 4.3 μM catalytic subunit was monophasic .(employing substrate in excess conditions), yielding an apparent value of Ki of .apprx.40 μM and a k2 value of .apprx.0.25 s-1. The low value of the observed Ki, together with the observation that **protein kinase** substrates inhibit the modification reactions, suggested strongly that the cysteine residue undergoing reaction is in the vicinity of the active site. By trypsin-catalyzed degradation and identification of the peptide segment modified by covalent attachment of the peptide portion of the radioactive analog, the single cysteine modified was identified as cysteine-198.

L8 ANSWER 12 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1982:506165 HCAPLUS Full-text
DOCUMENT NUMBER: 97:106165
TITLE: A kinetic study of interactions of (RP)- and (SP)-adenosine cyclic 3',5'-phosphorothioates with type II bovine cardiac muscle adenosine cyclic 3',5'-phosphate dependent **protein kinase**
AUTHOR(S): O'Brian, Catherine A.; Roczniak, Steven O.; **Bramson, H. Neal**; Baraniak, Janina; Stec, Wojciech J.; Kaiser, E. T.
CORPORATE SOURCE: Dep. Biochem., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Biochemistry (1982), 21(18), 4371-6
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The stereoselectivity of the cAMP-binding sites on the regulatory subunit of type II bovine cardiac muscle cAMP-dependent **protein kinase** was investigated by examining the interactions of the (RP)- and (SP)-adenosine cyclic 3',5'-phosphorothioate (cAMPS) diastereomers with these sites. Whereas activation of the **holoenzyme** and binding to the regulatory subunit of type II **protein kinase** were observed for both of these diastereomers, there were significant differences between the interactions of the cAMPS isomers with the **enzyme**. In particular, the SPisomer was more potent than the RPspecies, not only in the activation of reconstituted as well as directly isolated **holoenzyme**, but also in the inhibition of [3H]cAMP binding to the regulatory subunit. A marked preference for the binding of the SPisomer to site 2 in the regulatory subunit exists. H-bonding of a functional group on the regulatory subunit with preferential orientation toward the exocyclic O, rather than the S atom, of the thiophosphoryl residue may be involved in the observed selectivity of cAMPS binding and activation. In addition to these findings on the stereoselectivity of the binding of cAMPS to cAMP-dependent **protein kinase**, a method for the reconstitution of **holoenzyme** from the purified subunits without subjecting the regulatory protein to denaturing conditions was established.

L8 ANSWER 13 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1981:79297 HCAPLUS Full-text
DOCUMENT NUMBER: 94:79297
TITLE: Nuclear magnetic resonance studies of the conformation and kinetics of the peptide-substrate at the active site of bovine heart **protein kinase**
AUTHOR(S): Granot, Joseph; Mildvan, Albert S.; **Bramson, H.**

Neal; Thomas, N.; Kaiser, E. T.
CORPORATE SOURCE: Inst. Cancer Res., Fox Chase Cancer Cent., Philadelphia, PA, 19111, USA
SOURCE: Biochemistry (1981), 20(3), 602-10
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The conformation of the **enzyme**-bound peptide substrate on **protein kinase** from bovine heart has been studied by measurements of paramagnetic effects on the longitudinal relaxation rates of the protons of the peptide substrates, Leu-Arg-Arg-Ala-Ser-Leu-Gly and Leu-Arg-Arg-Tyr-Ser-Leu-Gly and the analog, Leu-Arg-Arg-Ala-Ala-Leu-Gly. Two metal ions which served sep. as reference points for distance measurements were Cr3+ at the metal activator site on the **enzyme**-bound nucleotide or Mn2+ at the inhibitory site which bridges the **enzyme** and the nucleotide. The relaxation rates of C-bound protons of the peptides measured at 100 and 360 MHz yielded distances from Cr3+ or Mn2+ to these protons (8.1 to ≥13 Å) which were used in the construction of mol. models employed in conformational studies. The measured distances were not compatible with α-helical and β-pleated-sheet conformations for the **enzyme**-bound heptapeptides. The 2 remaining classes of secondary structure, β-turns and coils, were consistent with the measured distances. Kinetic measurements, using a heptapeptide in which leucine-6 was replaced by proline-6, together with data from the literature were used to exclude the obligatory requirement for any β-turn possible within the heptapeptide studied, although an **enzymic** preference for a β2-5 or β3-6 turn is possible. Hence, if **protein kinase** has an absolute requirement for a specific secondary structure, then this structure must be a coil. The rate constant (.apprx.103 s-1) and kinetic parameters for the dissociation of the peptide from the **enzyme** complex were determined from the temperature dependence of the transverse relaxation rates. The rate constant for peptide binding (.apprx.107 M-1 s-1), determined from the transverse relaxation rate and the equilibrium constant, was smaller than expected for a diffusion-controlled reaction and may indicate that a conformational change occurs during peptide binding. In the phosphorylated seryl-peptide, the pKa of the phosphate (5.8) was found to be indistinguishable from that of phosphoserine or its di- and tripeptides, which argues against intramol interactions of the neg. charged phosphate with the pos. charged arginyl residues of the free phosphopeptide.

L8 ANSWER 14 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1980:633497 HCAPLUS Full-text
DOCUMENT NUMBER: 93:233497
TITLE: Development of a convenient spectrophotometric assay for peptide phosphorylation catalyzed by adenosine 3',5'-monophosphate dependent **protein kinase**
AUTHOR(S): **Bramson, H. Neal**; Thomas, Nancy; DeGrado, William F.; Kaiser, E. T.
CORPORATE SOURCE: Dep. Chem., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Journal of the American Chemical Society (1980), 102(23), 7156-7
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A reactive peptide substrate Leu-Arg-Arg-(o-NO2)Tyr-Ser-Leu-Gly (I) undergoes a spectral change at 430 nm upon phosphorylation at its serine residue catalyzed at pH 7.5 by the catalytic subunit of cAMP-dependent **protein kinase**. This peptide is the 1st substrate for which it is possible to monitor kinase action continuously spectrometrically, greatly facilitating kinetic and

mechanistic studies of the **enzyme**. The Km, peptide measured spectrometrically at pH 7.5 and 30.0° in the presence of 10 mM Mg2+ and 2 mM ATP was 40 + 10-6M with a kcat value of 3000 min-1. A brief study of the reactions of adenosine 5'-(1-thiotriphosphate) (ATPαS) A and B isomers by using I showed that the **protein kinase** has a marked preference for the Mg2+ complex of the B isomer which reacts at .apprx.13% of the rate at which Mg2+-ATP does. Apparently, the coordination of the metal ion to the α-position is important in the transition state for γ-phosphoryl transfer reactions catalyzed by **protein kinase**.

L8 ANSWER 15 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1980:491014 HCAPLUS Full-text
DOCUMENT NUMBER: 93:91014
TITLE: Magnetic resonance measurements of intersubstrate distances at the active site of **protein kinase** using substitution-inert cobalt(III) and chromium(III) complexes of adenosine 5'-(β,γ-methylenetriphosphate)
AUTHOR(S): Granot, Joseph; Mildvan, Albert S.; **Bramson, H. Neal**; Kaiser, E. T.
CORPORATE SOURCE: Inst. Cancer Res., Fox Chase Cancer Cent., Philadelphia, PA, 19111, USA
SOURCE: Biochemistry (1980), 19(15), 3537-43
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Co3+ and Cr3+ complexes of β,γ-methylene-ATP (AMPPCP), which are substitution-inert substrate analogs inactive in phosphoryl transfer reactions, were used in binding and structural studies of cAMP-dependent **protein kinase**. Dissociation consts. of **enzyme** complexes with Co(NH3)4AMPPCP and CrAMPPCP and with Mn2+, which binds at an inhibitory site, were determined by EPR and by proton relaxation rate enhancement techniques. Nuclear relaxation rate measurements at 100 and 360 MHz were used to determine the distance between Mn2+ and the β,γ-methylene protons of Co(NH3)4AMPPCP, yielding 7.4 Å in the absence of **enzyme** and 5.0 Å when both Mn2+ and Co(NH3)4AMPPCP were bound to the **enzyme**. The effect of the paramagnetic CrAMPPCP on the electron spin relaxation time of the **enzyme**-bound Mn2+ was used to calculate the distance between the 2 metal ions of 4.8 Å. This distance and the Mn2+-methylene distance are consistent with the previous finding that the inhibitory metal bridges the **enzyme** to the triphosphate chain of the **enzyme** -bound nucleotide (Granot, J., et al., 1979). From the paramagnetic effects on the relaxation rates of the protons of the peptide substrate Leu-Arg-Arg-Ala-Ser-Leu-Gly, distances from Mn2+ and Cr3+ to the serine methylene protons of 9.1 and 8.1 Å, resp., were calculated These and previous measurements were used to estimate a distance of 5.3 Å along the reaction coordinate between the γ-P of ATP and O of the OH of serine. This distance is 2 Å greater than that required for mol. contact. The mechanistic implications of these findings are discussed.

L8 ANSWER 16 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1980:142335 HCAPLUS Full-text
DOCUMENT NUMBER: 92:142335
TITLE: Stereochemical and kinetic studies on the action of the catalytic subunit of bovine cardiac muscle adenosine 3',5'-monophosphate dependent **protein kinase** using metal ion complexes of ATPβS
AUTHOR(S): Bolen, D. W.; Stingelin, Juerg; **Bramson, H.**

Neal; Kaiser, E. T.
CORPORATE SOURCE: Dep. Chem., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Biochemistry (1980), 19(6), 1176-82
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The phosphotransferase activity of bovine cardiac muscle **protein kinase** catalytic subunit was investigated by the use of metal ion complexes of ATPβS[adenosine 5'-O-(2-thiotriphosphate)] diastereomers (A and B) as donor substrates in peptide phosphorylation. MgATPβS A was used by the **enzyme** 500-fold faster than MgATPβS B, but CdIIATPβS B was preferred by .apprx.20-fold over CdIIATPβS A isomer. It is argued that the β,γ-bidentate complexes of MgATPβS A and of CdIIATPβS B, as well as the Δ isomer of CoIII(NH3)4ATP, have identical stereochem. about the β-P position and that this structure is preferentially recognized by the **enzyme**. A comparison of double-reciprocal kinetic plots suggested that the reaction with MgATPβS A proceeds by a ping-pong mechanism, whereas that with MgATP follows an ordered sequence. Further anal., including product inhibition and γ-32P exchange studies, showed that a ping-pong mechanism is not plausible for MgATPβS A and that, like MgATP, phosphorylation employing the A isomer probably proceeds by an ordered sequence. The **enzyme** uses MgATPβS A and MgATP as substrates in very much the same manner since the same kcat (.apprx.640 min -1) and Km (10.7 μM) values were obtained with either substrate. Kinetic differences between the reactions of the 2 nucleoside triphosphate substrates arise from different Km values for peptide and, presumably, from different dissociation consts. for the **enzyme**-Mg-nucleoside triphosphate complexes.

L8 ANSWER 17 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1979:553487 HCAPLUS Full-text
DOCUMENT NUMBER: 91:153487
TITLE: Specificity of bovine heart **protein kinase** for the Δ-stereoisomer of the metal-ATP complex
AUTHOR(S): Granot, Joseph; Mildvan, Albert S.; Brown, Eleanor M.; Kondo, Hiroki; **Bramson, H. Neal**; Kaiser, E. T.
CORPORATE SOURCE: Inst. Cancer Res., Fox Chase Cancer Cent., Philadelphia, PA, 19111, USA
SOURCE: FEBS Letters (1979), 103(2), 265-9
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English

AB When **protein kinase** catalytic subunit was incubated with β,γ-bidentate Co(NH3)4-ATP (either racemic mixture or resolved Δ-isomer), heptapeptide substrate, and Mn2+, approx. half of the racemic mixture (.apprx.44%) and the major part of the resolved Δ-isomer (.apprx.76%) were utilized. This supports the previous suggestion that the active **protein kinase** complex is an **enzyme**-nucleotide-metal complex. The rate with Co(NH3)4-ATP was very low (Vmax = 0.5 min-1) compared to that with Mg-ATP (Vmax = 2000 min-1) or Mn-ATP (Vmax = 510 min-1). The 1H NMR of the peptide substrate in the kinase reaction mixture with Co(NH3)4-ATP showed a decreased intensity of the Cβ-H2 resonance of the serine residue at 3.74 ppm with time and a new resonance increasing in magnitude at 0.07 ppm downfield. Thus, the serine residue of the peptide is phosphorylated by kinase and Co(III) remains coordinated to the phosphopeptide. The CD spectrum of a racemic Co(NH3)4-ATP mixture alone showed both pos. and neg. ellipticity, whereas the spectrum of a reaction

mixture showed only pos. ellipticity characterizing the inactive Λ-isomer of Co(NH3)4-ATP. Thus, the Δ-isomer is the preferred substrate.

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=> analyze l8 3 ct
L10          ANALYZE L8 3 CT :        9 TERMS

=> d
L10          ANALYZE L8 3 CT :        9 TERMS

TERM #    # OCC   # DOC   % DOC CT
------ ------- ------ ------ ---------------
     1        2      1 100.00 MASS SPECTROMETRY
     2        1      1 100.00 AFFINITY LABELING
     3        1      1 100.00 BIOTINYLATION
     4        1      1 100.00 ENZYME FUNCTIONAL SITES
     5        1      1 100.00 ENZYMES, BIOLOGICAL STUDIES
     6        1      1 100.00 IMMUNOBLOTTING
     7        1      1 100.00 LIQUID CHROMATOGRAPHY
     8        1      1 100.00 PROTEIN MOTIFS
     9        1      1 100.00 TRANSFORMING GROWTH FACTOR RECEPTORS
*********  END OF L10***
```

=> => d ind l8 3

L8 ANSWER 3 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
IC ICM C07D
CC 7-8 (Enzymes)
Section cross-reference(s): 9
ST **enzyme** inhibitor screening fluorosulfonylbenzoyl adenosine; **protein kinase** ATP binding site labeling biotinylated fluorosulfonylbenzoyl adenosine
IT Protein motifs
(ATP-binding site; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT Transforming growth factor receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TGF-β receptor, type I, kinase domain of; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT Immunoblotting
Mass spectrometry
(detecting **enzyme** inhibition with; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT Mass spectrometry
(liquid chromatog. combined with, detecting **enzyme** inhibition with; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT Liquid chromatography
(mass spectrometry combined with, detecting **enzyme** inhibition with; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT Affinity labeling
Biotinylation
Enzyme functional sites
(methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT **Enzymes**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT 62996-74-1, Staurosporine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(competes with FSBA labeling; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT 57454-44-1
RL: ARG (Analytical reagent use); RCT (Reactant); ANST (Analytical study); RACT (Reactant or reagent); USES (Uses)
(methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)
IT 57454-44-1DP, biotinylated 762241-22-5P 762241-23-6P

RL: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)
(methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)

IT 141349-86-2, CDK2 kinase 186709-18-2, **Protein kinase** ALK5 263554-79-6, **Protein kinase** ALK4 372092-80-3, **Protein kinase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)

IT 58-85-5, (+)-Biotin
RL: RCT (Reactant); RACT (Reactant or reagent)
(methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)

IT 56-65-5, 5'-ATP, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protected covalent modification of both ALK5 and ALK4 by FSBA; methods for identifying **enzyme** inhibitors: affinity labeling of ATP-binding site of **protein kinases** by biotinylated 5'-p-fluorosulfonylbenzoyl adenosine)

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=> d que stat l20

L11	1	SEA FILE=REGISTRY ABB=ON	FSBA/CN
L12	340	SEA FILE=HCAPLUS ABB=ON	L11 OR FSBA OR ?FLUOROSULFONYLBENZOYL?(3W)?ADENOSINE?
L13	1	SEA FILE=HCAPLUS ABB=ON	L12 AND ?BIOTINYLAT?
L14	243	SEA FILE=HCAPLUS ABB=ON	L12 AND ?BIND?
L15	99	SEA FILE=HCAPLUS ABB=ON	L14 AND ?KINASE?
L16	55	SEA FILE=HCAPLUS ABB=ON	L15 AND (?BIND? OR ?BOUND?)(3A)?KINASE?
L17	19	SEA FILE=HCAPLUS ABB=ON	L16 AND ?LYSINE?
L18	1	SEA FILE=HCAPLUS ABB=ON	L17 AND (?MASS?(W)?SPECT? OR ?PROTEASE?)
L19	20	SEA FILE=HCAPLUS ABB=ON	L13 OR L17 OR L18
L20	20	SEA FILE=HCAPLUS ABB=ON	L19 AND (PRD<20071008 OR PD<20071008)

=> d ibib abs l20 1-20

L20 ANSWER 1 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2007:315329 HCAPLUS Full-text
TITLE: Synthesis and characterization of 5'-p-fluorosulfonylbenzoyl-2'(or 3')-(biotinyl)adenosine as an activity-based probe for protein **kinases**
AUTHOR(S): Ratcliffe, Steven J.; Yi, Tracey; Khandekar, Sanjay S.
CORPORATE SOURCE: High Throughput Chemistry, Stevenage, GlaxoSmithKline, UK
SOURCE: Journal of Biomolecular Screening (**2007**), 12(1), 126-132
CODEN: JBISF3; ISSN: 1087-0571
PUBLISHER: Sage Publications
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Most of the **kinase** inhibitors that are approved for therapeutic uses or that are undergoing clin. trials are directed toward the ATP **binding** site of protein **kinases**. 5'- **Fluorosulfonylbenzoyl** 5'-**adenosine** (**FSBA**) is an activity-based probe (ABP) that covalently modifies a conserved **lysine** present in the nucleotide **binding** site of most **kinases**. Here the authors describe synthesis of **FSBA** derivs., 2'-biotinyl-**FSBA** and 3'-biotinyl-**FSBA** as **kinase** ABPs, and delineate a Western blot method to screen and validate ATP competitive protein **kinase** inhibitors using biotinyl-**FSBA** as a nonselective activity-based probe for protein **kinases**.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 2 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:1038103 HCAPLUS Full-text
DOCUMENT NUMBER: 144:246399
TITLE: A liquid chromatography/**mass spectrometry**-based method for the selection of ATP competitive **kinase** inhibitors
AUTHOR(S): Khandekar, Sanjay S.; Feng, Bingbing; Yi, Tracey; Chen, Susan; Laping, Nicholas; Bramson, Neal
CORPORATE SOURCE: Gene Expression and Protein Biochemistry, GlaxoSmithKline, King of Prussia, PA, USA
SOURCE: Journal of Biomolecular Screening (**2005**), 10(5), 447-455
CODEN: JBISF3; ISSN: 1087-0571
PUBLISHER: Sage Publications
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The currently approved **kinase** inhibitors for therapeutic uses and a number of **kinase** inhibitors that are undergoing clin. trials are directed toward the ATP (ATP) **binding** site of protein **kinases**. The 5'-**fluorosulfonylbenzoyl** 5'-**adenosine** (**FSBA**) is an ATP-affinity reagent that covalently modifies a conserved **lysine** present in the nucleotide-**binding** site of most **kinases**. The authors have developed a liquid chromatog./**mass spectrometry** -based method to monitor **binding** of ATP competitive protein **kinase** inhibitors using **FSBA** as a nonselective activity-based probe for protein **kinases**. Their method provides a general, rapid, and reproducible means to screen and validate selective ATP competitive inhibitors of protein **kinases**.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 3 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:799559 HCAPLUS Full-text
DOCUMENT NUMBER: 141:291247
TITLE: Methods for identifying enzyme inhibitors: affinity labeling of the ATP-binding site of protein kinases by **biotinylated** 5'-p-**fluorosulfonylbenzoyl adenosine**
INVENTOR(S): Bramson, Harold Neal; Glover, George I.; Khandekar, Sanjav; Ratcliffe, Steven John
PATENT ASSIGNEE(S): Smithkline Beecham Corporation, USA
SOURCE: PCT Int. Appl., 26 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 2004083175 A2 20040930 WO 2004-US8043 20040317 <--
WO 2004083175 A3 20050203
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
EP 1604021 A2 20051214 EP 2004-757519 20040317 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK
JP 2006520600 T 20060914 JP 2006-507244 20040317 <--
US 2006234327 A1 20061019 US 2005-549390 20050914 <--
PRIORITY APPLN. INFO.: US 2003-455374P P 20030317 <--
US 2003-487983P P 20030717 <--
WO 2004-US8043 W 20040317 <--

AB The present invention provides methods for identifying compds. that inhibit kinases. In addition, methods for profiling protein kinases are also provided. In addition, methods for determining the mode of action of kinase inhibitors are also provided. P-**Fluorosulfonylbenzoyl** 5'- **adenosine** (**FSBA**) is an ATP-affinity reagent that is effective in covalent modification of nucleotide-binding sites in a variety of protein kinases. For initial **FSBA** labeling studies, recombinantly expressed and purified kinase domain of the transforming growth factor (TGF)-β type I receptor (activin receptor-like kinase, ALK5) was used. Autoradiog. results indicated that **FSBA** labeled unheated ALK5 and CDK2 but did not label heat-denatured ALK5. Similar results were obtained for a panel of other kinases. The results indicate that **FSBA** and ATP compete for the same binding pocket in ALK5. Together the heat denaturation and ATP protection results indicated that **FSBA** has potential to be an activity-based probe for kinase profiling studies. Time-dependent reactions were performed to assess **FSBA** modification of kinases by LC/MS. ATP protected covalent modification of both ALK5 and ALK4 by **FSBA** in a concentration-dependent manner; most of the covalent modification was inhibited in the presence of 0.5 mM ATP. Likewise ATP protected labeling of CDK2 with **FSBA** in a concentration-dependent manner, although 5 mM ATP was necessary to block **FSBA** binding. **FSBA** binding to all enzymes tested was essentially blocked by the addition of 10 μM staurosporine. Synthesis of **biotinylated FSBA** [5'-p-**fluorosulfonylbenzoyl** -(2' or 3')-**biotinyladenosine**] is described. The results indicate that **biotinylated FSBA** modifies protein kinase as effectively as non-**biotinylated FSBA**. Similarly, these results indicate that LC/MS may be used to detect biotin- **FSBA** association with a kinase. One embodiment of the present invention is to provide a method for identifying a compound that inhibits an enzyme having an ATP binding site comprising contacting a, composition of the enzyme, an analyte capable of binding to the ATP binding site of the enzyme, and a test compound, and detecting whether the test compound inhibits the analyte from binding the ATP binding site of the enzyme.

L20 ANSWER 4 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:952728 HCAPLUS Full-text
DOCUMENT NUMBER: 138:250657
TITLE: The Nucleotide-**Binding** Site of Human Sphingosine **Kinase** 1
AUTHOR(S): Pitson, Stuart M.; Moretti, Paul A. B.; Zebol, Julia

R.; Zareie, Reza; Derian, Claudia K.; Darrow, Andrew L.; Qi, Jenson; D'Andrea, Richard J.; Bagley, Christopher J.; Vadas, Mathew A.; Wattenberg, Binks W.
CORPORATE SOURCE: Division of Human Immunology, Hanson Institute, Institute of Medical and Veterinary Science, Adelaide SA, 5000, Australia
SOURCE: Journal of Biological Chemistry (**2002**), 277(51), 49545-49553
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sphingosine **kinase** catalyzes the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in a number of agonist-driven cellular responses including mitogenesis, anti-apoptosis, and expression of inflammatory mols. Despite the importance of sphingosine **kinase**, very little is known regarding its structure or mechanism of catalysis. Moreover, sphingosine **kinase** does not contain recognizable catalytic or substrate-**binding** sites, based on sequence motifs found in other **kinases**. Here we have elucidated the nucleotide-**binding** site of human sphingosine **kinase** 1 (hSK1) through a combination of site-directed mutagenesis and affinity labeling with the ATP analog, **FSBA**. We have shown that Gly82 of hSK1 is involved in ATP **binding** since mutation of this residue to alanine resulted in an enzyme with an .apprx.45-fold higher Km(ATP). We have also shown that Lys103 is important in catalysis since an alanine substitution of this residue ablates catalytic activity. Furthermore, we have shown that this residue is covalently modified by **FSBA**. Our data, combined with amino acid sequence comparison, suggest a motif of SGDGX17-21K is involved in nucleotide **binding** in the sphingosine **kinases**. This motif differs in primary sequence from all previously identified nucleotide-**binding** sites. It does, however, share some sequence and likely structural similarity with the highly conserved glycine-rich loop, which is known to be involved in anchoring and positioning the nucleotide in the catalytic site of many protein **kinases**.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 5 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:299752 HCAPLUS Full-text
DOCUMENT NUMBER: 127:14773
TITLE: Pyridinyl imidazole inhibitors of p38 mitogen-activated protein **kinase** **bind** in the ATP site
AUTHOR(S): Young, Peter R.; McLaughlin, Megan M.; Kumar, Sanjay; Kassis, Shouki; Doyle, Michael L.; McNulty, Dean; Gallagher, Timothy F.; Fisher, Seth; McDonnell, Peter C.; Carr, Steven A.; Huddleston, Michael J.; Seibel, George; Porter, Terence G.; Livi, George P.; Adams, Jerry L.; Lee, John C.
CORPORATE SOURCE: Department of Molecular Immunology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA, 19406-0939, USA
SOURCE: Journal of Biological Chemistry (**1997**), 272(18), 12116-12121
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The site of action of a series of pyridinyl imidazole compds. that are selective inhibitors of p38 mitogen-activated protein **kinase** in vitro and

block proinflammatory cytokine production in vivo has been determined Using Edman sequencing, 125I-SB206718 was shown to cross-link to the nonphosphorylated Escherichia coli-expressed p38 **kinase** at Thr175, which is proximal to the ATP **binding** site. Titration calorimetric studies with E. coli-expressed p38 **kinase** showed that SB203580 **bound** with a stoichiometry of 1:1 and that **binding** was blocked by preincubation of p38 **kinase** with the ATP analog, **FSBA** (5'-[p-(fluorosulfonyl)benzoyl]adenosine), which covalently modifies the ATP **binding** site. The intrinsic ATPase activity of the nonphosphorylated enzyme was inhibited by SB203580 with a Km of 9.6 mM. Kinetic studies of active, phosphorylated yeast-expressed p38 **kinase** using a peptide substrate showed that SB203580 was competitive with ATP with a Ki of 21 nM and that **kinase** inhibition correlated with **binding** and biol. activity. Mutagenesis indicated that **binding** of 125I-SB206718 was dependent on the catalytic residues K53 and D168 in the ATP pocket. These findings indicate that the pyridinyl imidazoles act in vivo by inhibiting p38 **kinase** activity through competition with ATP and that their selectivity is probably determined by differences in nonconserved regions within or near the ATP **binding** pocket.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 6 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1996:452457 HCAPLUS Full-text
DOCUMENT NUMBER: 125:136316
TITLE: ATP and SH3 **binding** sites in the protein **kinase** of the large subunit of herpes simplex virus type 2 of ribonucleotide reductase (ICP10)
AUTHOR(S): Nelson, John W.; Zhu, Jia; Smith, Cynthia C.; Kulka, Michael; Aurelian, Laure
CORPORATE SOURCE: Virol./Immunol. Lab., Univ. Maryland Sch. Med., Baltimore, MD, 21201, USA
SOURCE: Journal of Biological Chemistry (**1996**), 271(29), 17021-17027
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) is a multifunctional protein. It consists of a ribonucleotide reductase and a serine/threonine protein **kinase** (PK) domain, which has three proline-rich motifs consistent with SH3-**binding** sites at positions 140, 149, and 396. The authors used site-directed mutagenesis to identify amino acids required for **kinase** activity and interaction with signaling proteins. Mutation of Lys176 or Lys259 reduced PK activity (5-8-fold) and **binding** of the 14C-labeled ATP analog p-**fluorosulfonylbenzoyl** 5'-**adenosine** (**FSBA**) but did not abrogate them. Enzymic activity and **FSBA binding** were abrogated by mutation of both Lys residues, suggesting that either one can **bind** ATP. Mutation of Glu209 (PK catalytic motif III) virtually abrogated **kinase** activity in the presence of Mg2+ or Mn2+ ions, suggesting that Glu209 functions in ion-dependent PK activity. ICP10 bound the adaptor protein Grb2 in vitro. Mutation of the ICP10 proline-rich motifs at positions 396 and 149 reduced Grb2 **binding** 20- and 2-fold, resp. **Binding** was abrogated by mutation of both motifs. Grb2 **binding** to wild type ICP10 was competed by a peptide for the Grb2 C-terminal SH3 motif, indicating that it involves the Grb2 C-terminal SH3.

L20 ANSWER 7 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1994:3528 HCAPLUS Full-text
DOCUMENT NUMBER: 120:3528

TITLE: Affinity labeling of smooth-muscle myosin light-chain **kinase** with 5'-[p-(fluorosulfonyl)benzoyl]adenosine
AUTHOR(S): Komatsu, Hideyuki; Ikebe, Mitsuo
CORPORATE SOURCE: Sch. Med., Case Western Res. Univ., Cleveland, OH, 44106, USA
SOURCE: Biochemical Journal (**1993**), 296(1), 53-8
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 5'-{P-(Fluorosulfonyl)[14C]benzoyl}adenosine (**FSBA**) was synthesized and used as a probe to study the ATP-**binding** site of smooth muscle myosin light-chain **kinase** (MLCK). **FSBA** modified both free MLCK and calmodulin/MLCK complex, resulting in inactivation of the **kinase** activity. Nearly complete protection of the calmodulin/MLCK complex against **FSBA** modification was obtained by addition of excess ATP whereas MLCK activity alone was lost in a dose-dependent manner even in the presence of excess ATP. These results suggest that **FSBA** modified ATP-**binding** sites and ATP-independent sites, and the latter sites are protected by calmodulin **binding**. The results also suggest that the ATP-**binding** site is accessible to the nucleotide substrate regardless of calmodulin **binding**. The **FSBA**-labeled MLCK was completely proteolyzed by α-chymotrypsin, and the 14C-labeled peptides were isolated and sequenced. The sequence of the labeled peptide was Ala-Gly-X-Phe, where X is the labeled residue. The sequence was compared with the known MLCK sequence, and the labeled residue was identified as **lysine**-548, which is located downstream of the GXGXXG motif conserved among ATP-utilizing enzymes.

L20 ANSWER 8 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1992:629090 HCAPLUS Full-text
DOCUMENT NUMBER: 117:229090
TITLE: Affinity labeling of the active site of brain phosphatidylinositol 4-**kinase** with 5'-**fluorosulfonylbenzoyl-adenosine**
AUTHOR(S): Scholz, Glen; Barritt, Greg J.; Kwok, Francis
CORPORATE SOURCE: Sch. Med., Flinders Univ. South Australia, Australia
SOURCE: European Journal of Biochemistry (**1992**), 210(2), 461-6
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 5'-p-**Fluorosulfonylbenzoyl-adenosine** (FSO2-BzAdo), an affinity labeling analog of ATP, was used to label the active site of sheep brain phosphatidylinositol 4-**kinase** (PtdIns 4- **kinase**). The incubation of PtdIns 4-**kinase** with concns. of FSO2BzAdo as low as 50 μM resulted in considerable inactivation of the enzyme (e.g. 55% less after 60 min with 50 μM FSO2BzAdo). The kinetics of inactivation of PtdIns 4-**kinase** by FSO2BzAdo suggest a two-step mechanism, in which a rapid reversible **binding** of FSO2BzAdo to the enzyme is followed by a covalent sulfonation step. The first-order rate constant (k2) for the inactivation of PtdIns 4- **kinase** was calculated to be 0.063 min-1, and the steady-state constant of inactivation (Ki) to be 200 μM. Preincubation of the enzyme with either ATP plus Mg2+, or PtdIns alone, prior to addition of FSO2BzAdo reduced the degree of inactivation of the enzyme; suggesting that FSO2BzAdo **binds** within the active site of PtdIns 4-**kinase**. Moreover, since ATP plus Mg2+ provided the greatest protection against inactivation, it is concluded that the main site of labeling of PtdIns 4- **kinase** by FSO2BzAdo is within the ATP-**binding** site of the enzyme. Results obtained from chemical modification expts., which employed pyridoxal 5'-phosphate and tetranitromethane, are consistent with a catalytically-essential **lysine** being

present within the ATP- **binding** site of PtdIns 4-**kinase**. Therefore, it is hypothesized that the activation of PtdIns 4-**kinase** by FSO2BzAdo may be due to the labeling of this **lysine** residue.

L20 ANSWER 9 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1989:71688 HCAPLUS Full-text
DOCUMENT NUMBER: 110:71688
TITLE: Affinity labeling of the ATP-**binding** site of type II calmodulin-dependent protein **kinase** by 5'-p-**fluorosulfonylbenzoyl adenosine**
AUTHOR(S): King, Marita M.; Shell, David J.; Kwiatkowski, Ann P.
CORPORATE SOURCE: Dep. Chem., Ohio State Univ., Columbus, OH, 43210, USA
SOURCE: Archives of Biochemistry and Biophysics (**1988**), 267(2), 467-73
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Modification of the type II calmodulin-dependent protein **kinase** by 5'-p-**fluorosulfonylbenzoyl adenosine** (**FSBA**) resulted in a time-dependent inactivation of the enzyme. The reaction followed pseudo-first-order kinetics and showed a nonlinear dependence on reagent concentration The rate of inactivation was sensitive to Mg2+- and calmodulin-induced conformational changes on the enzyme. However, the enhancing effects of these ligands were not additive; indeed, the kinetic parameters of the Mg2+-stimulated inactivation reaction with **FSBA** (Kinact = 2.4 mM; kmax = 0.12 min-1) were almost unaffected by the simultaneous addition of calmodulin (Kinact = 1.5 mM; kmax = 0.086 min-1). Protection from inactivation by **FSBA** was provided by Mg2+-ADP which is consistent with modification of the catalytic site. An anal. of the protective effect of Mg2+-ADP in the absence (Kd = 590 μM) and presence (Kd = 68 μM) of calmodulin demonstrated that **binding** of the modulator protein to the enzyme increases the affinity of the protein **kinase** for nucleotides. Modification by **FSBA** resulted in labeling of both tyrosine and **lysine** (Lys) residues but only labeling of Lys was decreased by Mg2+-ADP which is consistent with the hypothesis that a conserved Lys residue is important in nucleotide **binding** to the protein **kinase**. However, the kinetic results of the inactivation reaction suggest that this Lys is not involved in mediating the calmodulin-promoted increase in the affinity of the enzyme for Mg2+-nucleotide complexes.

L20 ANSWER 10 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1986:182412 HCAPLUS Full-text
DOCUMENT NUMBER: 104:182412
TITLE: Identification of tyrosine and **lysine** peptides labeled by 5'-p-**fluorosulfonylbenzoyl adenosine** in the active site of pyruvate **kinase**
AUTHOR(S): DeCamp, Dianne L.; Colman, Roberta F.
CORPORATE SOURCE: Dep. Chem., Univ. Delaware, Newark, DE, 19716, USA
SOURCE: Journal of Biological Chemistry (**1986**), 261(10), 4499-503
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nucleotide affinity label 5'-p-fluorosulfonylbenzoyladenosine (I) reacts at the active site of rabbit muscle pyruvate **kinase**, with irreversible inactivation occurring concomitant with incorporation of .apprx.1 mol of reagent/mol of enzyme subunit. Purified peptides were isolated from 70%

inactivated enzyme containing 0.7 mol of reagent/mol of enzyme subunit. Rabbit muscle enzyme labeled with radioactive I was digested with thermolysin. Nucleosidyl peptides were purified by chromatog. on phenylboronate-agarose and reverse-phase HPLC. After amino acid and N-terminal anal., the peptides were identified by comparison with the primary sequences of chicken and cat muscle enzyme. About 75% of the reagent incorporated was distributed equally among 3 O-(4- carboxybenzenesulfonyl)tyrosine (CBS-Tyr)-containing peptides: Leu-Asp-CBS-Tyr-Lys-Asn, Val-CBS-Tyr, and Leu-Asp-Asn-Ala-CBS-Tyr. These tyrosines were located in a 28-residue segment of the 530-amino acid sequence. The remainder of the incorporation was found in 2 Nε-(4-carboxybenzenesulfonyl)**lysine** (CBS-Lys)-containing peptides: Leu-CBS-Lys and Ala-CBS-Lys-Gly-Asp-Tyr-Pro. Modification in the presence of MnATP or MnADP resulted in a marked decrease in labeling of these peptides in proportion to the decreased inactivation. These modified residues are evidently located in the region of the catalytically functional nucleotide-**binding** site of pyruvate **kinase**.

L20 ANSWER 11 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1986:144603 HCAPLUS Full-text
DOCUMENT NUMBER: 104:144603
TITLE: A **lysine** in the ATP-**binding** site of P130gag-fps is essential for protein-tyrosine **kinase** activity
AUTHOR(S): Weinmaster, Geraldine; Zoller, Mark J.; Pawson, Tony
CORPORATE SOURCE: Dep. Microbiol., Univ. British Columbia, Vancouver, BC, V6T 1W5, Can.
SOURCE: EMBO Journal (**1986**), 5(1), 69-76
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The P130gag-fps transforming protein of Fujinami sarcoma virus (FSV) possesses tyrosine (Tyr)-specific protein **kinase** activity and autophosphorylates at Tyr-1073. Within the **kinase** domain of P130gag-fps is a putative ATP-**binding** site containing a **lysine** (Lys-950) homologous to **lysine** residues in cAMP-dependent protein **kinase** and p60v-src which **bind** the ATP analog, p-**fluorosulfonylbenzoyl**-5'-**adenosine**. FSV mutants in which the codon for Lys-950 was changed to codons for arginine or glycine encode metabolically stable but enzymically defective proteins which are unable to effect neoplastic transformation. **Kinase**-defective P130gag-fps containing arginine at residue 950 was normally phosphorylated at serine residues in vivo, suggesting that this amino acid substitution has a minimal effect on protein folding and processing. The inability of arginine to substitute for **lysine** at residue 950 suggests that the side-chain of Lys-950 is essential for P130gag-fps catalytic activity, probably by virtue of a specific interaction with ATP at the phosphotransfer active site. Tyr-1073 of the arginine-950 P130gag-fps mutant protein was not significantly autophosphorylated either in vitro or in vivo, but could be phosphorylated in trans by enzymically active P140gag-fps. Thus, Tyr-1073 apparently can be modified by intermol. autophosphorylation.

L20 ANSWER 12 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1985:200303 HCAPLUS Full-text
DOCUMENT NUMBER: 102:200303
TITLE: Identification of residues in the nucleotide **binding** site of the epidermal growth factor receptor/**kinase**
AUTHOR(S): Russo, Mark W.; Lukas, Thomas J.; Cohen, Stanley; Staros, James V.
CORPORATE SOURCE: Sch. Med., Vanderbilt Univ., Nashville, TN, 37232, USA

SOURCE: Journal of Biological Chemistry (**1985**), 260(9), 5205-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The epidermal growth factor (EGF) receptor/**kinase** was purified from A431 membrane vesicles affinity labeled with the ATP analog 5'-p-**fluorosulfonylbenzoyl**[8-14C]**adenosine**. The resulting purified, affinity-labeled receptor/**kinase** preparation was reduced and carboxymethylated, and subjected to tryptic digestion. From this digest, the tryptic peptide containing the major site of labeling by the ATP analog was isolated and sequenced. The sequence of this peptide is Ile-Pro-Val-Ala-Ile-X-Glu-Leu, where X corresponds to **lysine**-721 of the derived sequence of the EGF receptor/**kinase**.

L20 ANSWER 13 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1985:20314 HCAPLUS Full-text
DOCUMENT NUMBER: 102:20314
TITLE: Identification of the active site of the transforming protein of Rous sarcoma virus
AUTHOR(S): Kamps, Mark P.; Sefton, Bartholomew M.
CORPORATE SOURCE: Dep. Chem., Univ. California, San Diego, La Jolla, CA, 92093, USA
SOURCE: Cancer Cells (1984-1989) (**1984**), 2(Oncog. Viral Genes), 53-9
CODEN: CACEEG; ISSN: 0743-2194
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ATP analog, **FSBA** (p-fluorosulfonylbenzyl-5'-adenosine), was used to locate the ATP-**binding** site of p60src. **FSBA** reacted with **lysine**-295 and inactivated the **kinase** activity of p60src by this covalent modification. Therefore, **lysine**-295 and the residues that adjoin it comprise part of the active site of p60src. By virtue of its pos. charge, **lysine**-295 itself may function as an essential residue for **binding** ATP. All 4 tyrosine protein **kinases** whose sequences are known, and the cAMP-dependent serine protein **kinase**, have sequence homol. When aligned, all 5 proteins contain a **lysine** at exactly the same position as **lysine**-295 of p60src. The homologous **lysine** in the cAMP-dependent protein **kinase** also reacts with **FSBA**. Thus, it appears that the ATP-**binding** regions contained within the active sites of both serine and tyrosine **kinases** are functionally very similar.

L20 ANSWER 14 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1984:546769 HCAPLUS Full-text
DOCUMENT NUMBER: 101:146769
TITLE: Direct evidence that oncogenic tyrosine **kinases** and cyclic AMP-dependent protein **kinase** have homologous ATP-**binding** sites
AUTHOR(S): Kamps, Mark P.; Taylor, Susan S.; Sefton, Bartholomew M.
CORPORATE SOURCE: Mol. Biol. Virol. Lab., Salk Inst., San Diego, CA, 92138, USA
SOURCE: Nature (London, United Kingdom) (**1984**), 310(5978), 589-92
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB It was demonstrated that the ATP analog, p-**fluorosulfonylbenzoyl** 5'-**adenosine** (**FSBA**), inactivates the tyrosylprotein **kinase** activity of pp60src, the transforming protein of Rous sarcoma virus, by reacting with **lysine** 295. When aligned for maximum sequence identity, **lysine**-295 of pp60src and the **lysine** in the catalytic subunit of cAMP-dependent protein **kinase**, which also reacts specifically with **FSBA**, are precisely superimposed. This functional homol. is strong evidence that the protein **kinases**, irresp. of amino acid substrate specificity, comprise a single divergent gene family.

L20 ANSWER 15 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1984:506455 HCAPLUS Full-text
DOCUMENT NUMBER: 101:106455
TITLE: Adenosine cyclic 3',5'-monophosphate dependent protein **kinase**: nucleotide **binding** to the chemically modified catalytic subunit
AUTHOR(S): Bhatnagar, Deepak; Hartl, F. Thomas; Roskoski, Robert, Jr.; Lessor, Ralph A.; Leonard, Nelson J.
CORPORATE SOURCE: Med. Cent., Louisiana State Univ., New Orleans, LA, 70119, USA
SOURCE: Biochemistry (**1984**), 23(19), 4350-7
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 5'-[p-(Fluorosulfonyl)benzoyl]adenosine (**FSBA**) inactivates the catalytic (C) subunit of cAMP-dependent protein **kinase** isolated from bovine cardiac muscle by covalent modification of **lysine** -71, whereas 7-chloro-4-nitro-2,1,3-benzoxadiazole (NBD-Cl) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) react with cysteines-199 and -343 to inactivate the enzyme. All 3 of these reagents have been postulated to modify residues at or near the active site of the catalytic subunit. ATP (2 mM) in the presence of excess Mg2+ (10 mM) protected the enzyme against inactivation by these reagents. AMP did not afford any protection, but adenosine slightly decreased the rate of inactivation. The specific effects of covalent modification of **lysine**-71 and cysteines-199 and -343 on nucleotide **binding** were characterized by fluorescence polarization titrns. with lin-benzoadenine nucleotides as fluorescent ligands. lin-Benzoadenosine was a competitive inhibitor of the catalytic subunit with respect to ATP with a Ki (38 μM) similar to the Ki for adenosine (35 μM). This value agreed well with the dissociation constant (Kd) (32 μM) for adenosine determined by fluorescence polarization titrns. lin-Benzo-ADP was previously shown to be a competitive inhibitor with respect to ATP and lin-benzo-ATP was a substrate for the phosphotransferase activity of the protein **kinase**. Modification by **FSBA**, NBD-Cl, or DTNB resulted in >85% inhibition of phosphotransferase activity as well as complete inhibition of lin-benzo-ADP and lin-benzo-ATP **binding** in the presence of 10 mM Mg2+. lin-Benzoadenosine, on the other hand, bound to the enzyme with the same Kd and stoichiometry (1 mol/mol) as it did to the unmodified enzyme (Kd = 26-35 μM). Whereas all effectively displaced lin-benzoadenosine bound to the unmodified catalytic subunit, AMP, but not MgATP or MgADP, displaced the fluorescent probe from enzyme modified with NBD-Cl, DTNB, or **FSBA**. The Kd for AMP (804-856 μM), however, was 25% greater for the modified enzyme. These reagents, which are thought to modify residues that are at or near the active site of the catalytic subunit, inactivated the enzyme by inhibiting nucleotide **binding**. This effect involved the region on the C subunit complementary to the β- and γ-phosphates of the ATP mol. as compared to the region complementary to the α-phosphate of the nucleotide **binding** portion of the C subunit.

L20 ANSWER 16 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1982:419795 HCAPLUS Full-text
DOCUMENT NUMBER: 97:19795
TITLE: Nucleotide-**binding** sites and structural domains of cAMP-dependent protein **kinases**
AUTHOR(S): Taylor, Susan S.; Kerlavage, Anthony R.; Zoller, M. J.; Nelson, N. C.; Potter, R. L.
CORPORATE SOURCE: Dep. Chem., Univ. California, La Jolla, CA, 92093, USA
SOURCE: Cold Spring Harbor Conferences on Cell Proliferation (**1981**), 8(Protein Phosphorylation, Book A), 3-18
CODEN: CSHCAL; ISSN: 0097-5230
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Structural domains of porcine muscle cAMP-dependent protein **kinase** (I) catalytic (C) and regulatory (RI and RII) subunits were studied by using proteolytic fragments and by affinity labeling with ATP and cAMP analogs. With the exception of a possible site for RII dimer interaction by SS bonding in the N-terminal domain, the 2 R subunits are similar in structure. Structural homol. includes the cAMP-**binding** site and the C subunit recognition site. The latter may be the major site for R-C subunit interaction and for autophosphorylation of the RII subunits. The R subunit cAMP-**binding** sites have high specificity for 8-azido-cAMP affinity labeling. The C subunits of I types I and II are highly conserved. Modification with ATP analogs (including p- **fluorosulfonylbenzoyl** 5'-**adenosine**) and group-specific reagents indicate 3 essential residues associated with ATP **binding**: cysteine residues 198 and 342 and **lysine**-71.

L20 ANSWER 17 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1982:64811 HCAPLUS Full-text
DOCUMENT NUMBER: 96:64811
TITLE: Amino acid sequence at the ATP **binding** site of cGMP-dependent protein **kinase**
AUTHOR(S): Hashimoto, Eikichi; Takio, Koji; Krebs, Edwin G.
CORPORATE SOURCE: Sch. Med., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: Journal of Biological Chemistry (**1981**), 257(2), 727-33
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The amino acid sequence at the ATP **binding** site on the cGMP-dependent protein **kinase** has been determined For this determination, the enzyme was labeled covalently by 5'-p-fluorosulfonyl[14C]benzoyladenos ine and fragmented using CNBr or digested by trypsin after succinylation. The 14C-labeled peptides were purified by gel filtration and high-performance liquid chromatog. The amino acid sequence around the site was determined and a **lysine** residue was found to be modified by the affinity reagent. When this sequence was compared with that of the ATP- **binding** site of the catalytic subunit of cAMP-dependent protein **kinase**, a high degree of structural homol. was observed for this site in the 2 proteins.

L20 ANSWER 18 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1981:583063 HCAPLUS Full-text
DOCUMENT NUMBER: 95:183063
TITLE: Affinity labeling of cAMP-dependent protein **kinase** with p-**fluorosulfonylbenzoyl** **adenosine**. Covalent modification of **lysine** 71

AUTHOR(S): Zoller, Mark J.; Nelson, Norman C.; Taylor, Susan S.
CORPORATE SOURCE: Dep. Chem., Univ. California, La Jolla, CA, 92093, USA
SOURCE: Journal of Biological Chemistry (**1981**), 256(21), 10837-42
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB p-**Fluorosulfonylbenzoyl**-5'-**adenosine** (I) was shown previously to be an irreversible inhibitor of the catalytic subunit of cAMP-dependent protein **kinase** II from porcine skeletal muscle. The catalytic subunit of porcine heart cAMP-dependent protein **kinase** was also inhibited following incubation with I-14C and inhibition was shown to result from the stoichiometric, covalent modification of a single **lysine** residue. The amino acid sequence in an extended region around the carboxybenzenesulfonyl **lysine** was elucidated by characterizing both tryptic and CNBr peptides containing the 14C-modified residue. The covalently modified residue corresponded to **lysine**-71 in the overall polypeptide chain. Homologies with bovine heart catalytic subunit and to a site modified by I in **phosphofructokinase** were considered.

L20 ANSWER 19 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1981:79343 HCAPLUS Full-text
DOCUMENT NUMBER: 94:79343
TITLE: Affinity labeling of the ATP **binding** site of bovine lung cyclic GMP-dependent protein **kinase** with 5'-p-fluorosulfonylbenzoyladenosin e
AUTHOR(S): Hixson, Craig S.; Krebs, Edwin G.
CORPORATE SOURCE: Sch. Med., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: Journal of Biological Chemistry (**1981**), 256(3), 1122-7
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bovine lung cyclic GMP-dependent protein **kinase** (I) was covalently labeled with the ATP analog, 5'-p-fluorosulfonylbenzoyladenosin e (II). The inactivation reaction was pseudo-1st-order. The rate of I sulfonation exhibited saturation kinetics, indicative of a rapid reversible **binding** of II to prior to enzyme modification. I could be protected by MgATP, MgADP, and Mg-adenylylimidodiphosphate but not by a synthetic peptide substrate. Cyclic GMP when **bound** to the **kinase** did not influence the rate of labeling. II demonstrated competitive inhibition with respect to MgATP; the Ki was found to be 0.82 mM. Mg2+ and Co2+, when included in the reaction mixture, accelerated the inactivation rate up to several-fold. Addition of basic polypeptides, such as mixed histone, protamine sulfate, or poly-L-**lysine** HBr, also markedly accelerated the sulfonation rate. Inactivation of I by II-3H resulted in a linear relation between the residual phosphotransferase activity and the incorporation of up to 0.9 mol II/mol monomer.

L20 ANSWER 20 OF 20 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1979:570776 HCAPLUS Full-text
DOCUMENT NUMBER: 91:170776
TITLE: Affinity labeling of the nucleotide **binding** site of the catalytic subunit of cAMP-dependent protein **kinase** using p-fluorosulfonyl-[14C]-benzoyl 5'-adenosine. Identification of a modified **lysine** residue
AUTHOR(S): Zoller, Mark J.; Taylor, Susan S.
CORPORATE SOURCE: Dep. Chem., Univ. California, La Jolla, CA, 92093, USA

SOURCE: Journal of Biological Chemistry (**1979**), 254(17), 8363-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Treatment of the catalytic subunit of cyclic AMP (cAMP)-dependent protein **kinase** II from porcine skeletal muscle with p- **fluorosulfonylbenzoyl** 5'-**adenosine** (I) resulted in complete inhibition of protein **kinase** activity. Both MgATP and MgADP at concns. approx. equal to that of I afforded complete protection against inactivation. Free ATP, ADP, and AMP were considerably less effective in protecting against inactivation by I. At high concns. (100 mM), Mg2+ alone significantly enhanced the rate of inhibition. Inhibition with I followed pseudo-1st order kinetics. The pseudo-1st order rate constant for inactivation showed saturation kinetics, indicating reversible **binding** of the reagent to the enzyme prior to inactivation. The incorporation of 1 mol I-14C/mol catalytic subunit was sufficient to inhibit enzymic activity completely. Following tryptic digestion of the 14C covalently modified protein, a single radioactive peptide was isolated. This peptide was purified by ion exchange chromatog. before and after mild alkaline hydrolysis which specifically cleaved the ester bond of the **fluorosulfonylbenzoyladenosine** label, releasing free **adenosine** and generating an extra neg. charge on the carboxybenzoylsulfonyl-labeled peptide. The purified peptide migrated as a single spot following both paper electrophoresis at pH 6.5 and 2-dimensional thin-layer peptide mapping. The labeled amino acid residue was identified as **carboxybenzoylsulfonyllysine**, and the amino acid composition of the peptide was determined The N-terminal residue of the peptide was identified by dansylation as glutamate, whereas treatment with carboxypeptidases A and B released only a single **lysine** residue.

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=> d que stat l24
L11           1 SEA FILE=REGISTRY ABB=ON  FSBA/CN
L12         340 SEA FILE=HCAPLUS ABB=ON  L11 OR FSBA OR ?FLUOROSULFONYLBENZOYL?
                (3W)?ADENOSINE?
L13           1 SEA FILE=HCAPLUS ABB=ON  L12 AND ?BIOTINYLAT?
L14         243 SEA FILE=HCAPLUS ABB=ON  L12 AND ?BIND?
L15          99 SEA FILE=HCAPLUS ABB=ON  L14 AND ?KINASE?
L16          55 SEA FILE=HCAPLUS ABB=ON  L15 AND (?BIND? OR ?BOUND?)(3A)?KINASE
                ?
L17          19 SEA FILE=HCAPLUS ABB=ON  L16 AND ?LYSINE?
L18           1 SEA FILE=HCAPLUS ABB=ON  L17 AND (?MASS?(W)?SPECT? OR ?PROTEASE
                ?)
L19          20 SEA FILE=HCAPLUS ABB=ON  L13 OR L17 OR L18
L21          15 SEA L19
L22           8 DUP REMOV L21 (7 DUPLICATES REMOVED)
L23           1 SEA FILE=WPIDS ABB=ON  L13 OR L17 OR L18
L24           9 DUP REMOV L22 L23 (0 DUPLICATES REMOVED)


=> d ibib abs l24 1-9

L24  ANSWER 1 OF 9     MEDLINE on STN
ACCESSION NUMBER:    2007106923     MEDLINE  Full-text
DOCUMENT NUMBER:     PubMed ID: 17166825
TITLE:               Synthesis and characterization of 5'-p-
                     fluorosulfonylbenzoyl-2' (or 3')-(biotinyl)adenosine as an
                     activity-based probe for protein kinases.
AUTHOR:              Ratcliffe Steven J; Yi Tracey; Khandekar Sanjay S
CORPORATE SOURCE:    High Throughput Chemistry, Stevenage, GlaxoSmithKline, UK.
SOURCE:              Journal of biomolecular screening : the official journal of
                     the Society for Biomolecular Screening, (2007 Feb) Vol. 12,
                     No. 1, pp. 126-32.  Electronic Publication: 2006-12-08.
                     Journal code: 9612112. ISSN: 1087-0571.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200703
ENTRY DATE:          Entered STN: 21 Feb 2007
                     Last Updated on STN: 28 Mar 2007
                     Entered Medline: 27 Mar 2007
AB   Most of the kinase inhibitors that are approved for therapeutic uses or that
     are undergoing clinical trials are directed toward the adenosine triphosphate
     (ATP) binding site of protein kinases. 5'-Fluorosulfonylbenzoyl 5'-adenosine
     (FSBA) is an activitybased probe (ABP) that covalently modifies a conserved
     lysine present in the nucleotide binding site of most kinases.  Here the
     authors describe synthesis of FSBA derivatives, 2'-biotinyl-FSBA and 3'-
     biotinyl- FSBA as kinase ABPs, and delineate a Western blot method to screen
     and validate ATP competitive protein kinase inhibitors using biotinyl-FSBA as
     a nonselective activity-based probe for protein kinases.


L24  ANSWER 2 OF 9     MEDLINE on STN
ACCESSION NUMBER:    2005427665     MEDLINE  Full-text
DOCUMENT NUMBER:     PubMed ID: 16093554
TITLE:               A liquid chromatography/mass spectrometry
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-based method for the selection of ATP competitive **kinase** inhibitors.
AUTHOR: Khandekar Sanjay S; Feng Bingbing; Yi Tracey; Chen Susan; Laping Nicholas; Bramson Neal
CORPORATE SOURCE: Gene Expression and Protein Biochemistry, GlaxoSmithKline, King of Prussia, PA 19406, USA.. sanjay.khandekar-1@gsk.com
SOURCE: Journal of biomolecular screening : the official journal of the Society for Biomolecular Screening, (2005 Aug) Vol. 10, No. 5, pp. 447-55.
Journal code: 9612112. ISSN: 1087-0571.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200510
ENTRY DATE: Entered STN: 15 Aug 2005
Last Updated on STN: 25 Oct 2005
Entered Medline: 24 Oct 2005

AB The currently approved **kinase** inhibitors for therapeutic uses and a number of **kinase** inhibitors that are undergoing clinical trials are directed toward the adenosine triphosphate (ATP) **binding** site of protein **kinases**. The 5'-**fluorosulfonylbenzoyl** 5'-**adenosine** (**FSBA**) is an ATP-affinity reagent that covalently modifies a conserved **lysine** present in the nucleotide-**binding** site of most **kinases**. The authors have developed a liquid chromatography/ **mass spectrometry**-based method to monitor **binding** of ATP competitive protein **kinase** inhibitors using **FSBA** as a nonselective activity-based probe for protein **kinases**. Their method provides a general, rapid, and reproducible means to screen and validate selective ATP competitive inhibitors of protein **kinases**.

L24 ANSWER 3 OF 9 WPIDS COPYRIGHT 2007 THE THOMSON CORP on STN
ACCESSION NUMBER: 2004-728449 [71] WPIDS
DOC. NO. CPI: C2004-255943 [71]
DOC. NO. NON-CPI: N2004-576953 [71]
TITLE: Identification of inhibitors of enzyme that has adenosine triphosphate **binding** site involves detecting whether test compound inhibits analyte from **binding** the site of enzyme during incubation
DERWENT CLASS: B02; B04; D16; S03
INVENTOR: BRAMSON H N; GLOVER G I; KHANDEKAR S; RATCLIFFE S J
PATENT ASSIGNEE: (SMIK-C) SMITHKLINE BEECHAM CORP; (BRAM-I) BRAMSON H N; (GLOV-I) GLOVER G I; (KHAN-I) KHANDEKAR S; (RATC-I) RATCLIFFE S J
COUNTRY COUNT: 107

PATENT INFO ABBR.:

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AN 2004-728449 [71] WPIDS

AB WO 2004083175 A2 UPAB: 20060203

NOVELTY - Identification (M1) of a compound that inhibits an enzyme having an adenosine triphosphate (ATP) **binding** site, involves contacting a composition comprising the enzyme and an analyte with a test compound; and detecting whether the test compound inhibits the analyte from **binding** the ATP **binding** site.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) identification (M2) of a protein **kinase** that has ATP **binding** site involving contacting a composition comprising the protein **kinase** with the analyte, and detecting whether the analyte **binds** to the **kinase**; (2) para-**fluorosulfonylbenzoyl** 5'-**adenosine** (**FSBA**) of formula (I) as analyte in (M1) and (M2); and (3) preparation of (I) involving either process (A): dissolving (+)-biotin, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate in dry N,N-dimethylformamide (DMF) and heating followed by cooling; adding di-isopropylcarbodiimide to the cooled solution; adding the solution to an ice-cold solution of 5'-(4- **fluorosulfonylbenzoyl**)**adenosine** in dry DMF and N,N-diisopropylethylamine; and adding N,N-dimethylaminopyridine (DMAP) in dry DMF to the solution and warming slowly; or process (B): dissolving (+)-Biotin, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, N-hydroxybenzotriazole.H2O, DMAP and 5'-(4-**fluorosulfonylbenzoyl**)**adenosine** in dry DMF; chilling the solution; adding N,N-diisopropylethylamine to the solution; and warming the solution to room temperature and stirring. R1, R2 = H or biotinoyl.

USE - For identification of competitive inhibitors of **FSBA** as inhibitor of enzyme (e.g. **kinase**) that has adenosine triphosphate **binding** site, and also for identifying mode of action of the inhibitor and its selectivity for enzymes (claimed).

ADVANTAGE - The method provides general, rapid and reproducible way for screening ATP-competitive inhibitors of recombinant as well as purified enzymes e.g. **kinase**.

L24 ANSWER 4 OF 9 MEDLINE on STN
ACCESSION NUMBER: 2002731982 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 12393916
TITLE: The nucleotide-**binding** site of human sphingosine **kinase** 1.
AUTHOR: Pitson Stuart M; Moretti Paul A B; Zebol Julia R; Zareie Reza; Derian Claudia K; Darrow Andrew L; Qi Jenson; D'Andrea Richard J; Bagley Christopher J; Vadas Mathew A; Wattenberg Binks W

CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide SA 5000, Australia.. stuart.pitson@imvs.sa.gov.asu
SOURCE: The Journal of biological chemistry, (2002 Dec 20) Vol. 277, No. 51, pp. 49545-53. Electronic Publication: 2002-10-18.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 27 Dec 2002
Last Updated on STN: 14 Feb 2003
Entered Medline: 12 Feb 2003

AB Sphingosine **kinase** catalyzes the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in a number of agonist-driven cellular responses including mitogenesis, anti-apoptosis, and expression of inflammatory molecules. Despite the importance of sphingosine **kinase**, very little is known regarding its structure or mechanism of catalysis. Moreover, sphingosine **kinase** does not contain recognizable catalytic or substrate-**binding** sites, based on sequence motifs found in other **kinases**. Here we have elucidated the nucleotide-**binding** site of human sphingosine **kinase** 1 (hSK1) through a combination of site-directed mutagenesis and affinity labeling with the ATP analogue, **FSBA**. We have shown that Gly(82) of hSK1 is involved in ATP **binding** since mutation of this residue to alanine resulted in an enzyme with an approximately 45-fold higher K(m)((ATP)). We have also shown that Lys(103) is important in catalysis since an alanine substitution of this residue ablates catalytic activity. Furthermore, we have shown that this residue is covalently modified by **FSBA**. Our data, combined with amino acid sequence comparison, suggest a motif of SGDGX(17-21)K is involved in nucleotide **binding** in the sphingosine **kinases**. This motif differs in primary sequence from all previously identified nucleotide-**binding** sites. It does, however, share some sequence and likely structural similarity with the highly conserved glycine-rich loop, which is known to be involved in anchoring and positioning the nucleotide in the catalytic site of many protein **kinases**.

L24 ANSWER 5 OF 9 MEDLINE on STN
ACCESSION NUMBER: 89000819 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 2844270
TITLE: Identification of the AMP **binding** sites of rabbit phosphofructo-1-**kinase** isozymes B and C.
AUTHOR: Valaitis A P; Kwiatkowska D; Krishnaraj R; Kemp R G
CORPORATE SOURCE: Department of Biological Chemistry and Structure, University of Health Sciences, Chicago Medical School, IL.
CONTRACT NUMBER: DK 19912 (NIDDK)
DK 26564 (NIDDK)
SOURCE: Biochimica et biophysica acta, (1988 Oct 12) Vol. 956, No. 3, pp. 232-42.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 20 Apr 2002

Entered Medline: 16 Nov 1988

AB Rabbit liver phosphofructo-1-**kinase**, designated isozyme B, and rabbit brain **phosphofructokinase**, which contains all three isozymes as heteropolymers, have been modified by [14C]fluorosulfonylbenzoyladenosine (FSBAdo). Several lines of evidence supported modification at the **binding** site for AMP. The modification proceeded to the extent of 2 to 4 mol of reagent incorporated per mol of tetramer, and AMP protected against the reaction. The kinetic properties of modified isozymes A and B and of modified brain **phosphofructokinase** were examined and compared to their unmodified forms. It was observed that modification greatly diminished ATP inhibition of all of the isozymes. Furthermore, equilibrium **binding** studies of modified **phosphofructokinase** B showed a greatly diminished capacity and affinity for cyclic AMP. Cyclic AMP had little or no influence on the properties of modified A isozyme or brain **phosphofructokinase**, but was capable of further deinhibiting modified B isozyme, apparently at sites remaining unmodified by FSBAdo. **Phosphofructokinase** B, modified by radiolabeled FSBAdo, was digested by trypsin, and the digest separated by high-pressure liquid chromatography. The labeled peptide was isolated and sequenced to provide the sequence: Asn-Tyr-Gly-Thr-Lys-Leu-Gly-Val-Lys, with the **lysine** in the fifth position being the site of modification. To isolate isozyme C, a monoclonal antibody to this isozyme was produced by injecting purified rabbit brain **phosphofructokinase** into mice, and subsequently selecting for those clones that recognized brain **phosphofructokinase** but not purified **phosphofructokinases** A and B. The selected monoclonal was specific for native rabbit isozyme C and would not recognize mouse or rat brain **phosphofructokinases**. Linking the antibody to an inert phase provided an efficient means of purifying rabbit isozyme C from rabbit brain. The enzyme so recovered retained little of its original activity, but the method provided a simple technique for the preparation of enzyme for protein chemistry studies. The modified C isozyme was isolated on the immuno-affinity column and digested with trypsin. A tryptic peptide bearing the label was isolated and sequenced to provide the structure: Asn-Phe-Gly-Thr-Lys-Ile-Ser-Ala- Arg, with position 5 being the site of modification. The sequences of isozymes B and C are homologous to the site of modification of the A isozyme by FSBAdo.

L24 ANSWER 6 OF 9 MEDLINE on STN

ACCESSION NUMBER:	86168146 MEDLINE Full-text
DOCUMENT NUMBER:	PubMed ID: 3082867
TITLE:	Identification of tyrosine and **lysine** peptides labeled by 5'-p-**fluorosulfonylbenzoyl adenosine** in the active site of pyruvate **kinase**.
AUTHOR:	DeCamp D L; Colman R F
SOURCE:	The Journal of biological chemistry, (1986 Apr 5) Vol. 261, No. 10, pp. 4499-503. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY:	United States
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	198605
ENTRY DATE:	Entered STN: 21 Mar 1990 Last Updated on STN: 20 Apr 2002 Entered Medline: 9 May 1986

AB The nucleotide affinity label 5'-p-**fluorosulfonylbenzoyl adenosine** reacts at the active site of rabbit muscle pyruvate **kinase**, with irreversible inactivation occurring concomitant with incorporation of about 1 mol of reagent/mol of enzyme subunit (Annamalai, A. E., and Colman, R. F. (1981) J.

Biol. Chemical 256, 10276-10283). Purified peptides have now been isolated from 70% inactivated enzyme containing 0.7 mol of reagent/mol of enzyme subunit. Rabbit muscle enzyme labeled with radioactive 5'-p-**fluorosulfonylbenzoyl adenosine** was digested with thermolysin. Nucleosidyl peptides were purified by chromatography on phenylboronate-agarose and reverse-phase high performance liquid chromatography. After amino acid and N-terminal analysis, the peptides were identified by comparison with the primary sequences of chicken and cat muscle enzyme. About 75% of the reagent incorporated was distributed equally among three O-(4-carboxybenzenesulfonyl)tyrosine-containing peptides: Leu-Asp-CBS-Tyr-Lys-Asn, Val-CBS-Tyr, and Leu-Asp-Asn-Ala-CBS-Tyr. These tyrosines are located in a 28-residue segment of the 530-amino acid sequence. The remainder of the incorporation was found in two N epsilon-(4-carboxybenzenesulfonyl)**lysine**-containing peptides. Leu-CBS-Lys and Ala-CBS-Lys-Gly-Asp-Tyr-Pro. Modification in the presence of MnATP or MnADP resulted in a marked decrease in labeling of these peptides in proportion to the decreased inactivation. It is suggested that these modified residues are located in the region of the catalytically functional nucleotide **binding** site of pyruvate **kinase**.

L24 ANSWER 7 OF 9 MEDLINE on STN
ACCESSION NUMBER: 85023325 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 6487604
TITLE: Adenosine cyclic 3',5'-monophosphate dependent protein **kinase**: nucleotide **binding** to the chemically modified catalytic subunit.
AUTHOR: Bhatnagar D; Hartl F T; Roskoski R Jr; Lessor R A; Leonard N J
CONTRACT NUMBER: GM-05829 (NIGMS)
NS-15994 (NINDS)
SOURCE: Biochemistry, (1984 Sep 11) Vol. 23, No. 19, pp. 4350-7.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198412
ENTRY DATE: Entered STN: 20 Mar 1990
Last Updated on STN: 20 Apr 2002
Entered Medline: 14 Dec 1984

AB 5'-[p-(Fluorosulfonyl)benzoyl]adenosine (**FSBA**) inactivates the catalytic subunit of the adenosine cyclic 3',5'-monophosphate dependent protein **kinase** isolated from bovine cardiac muscle by covalent modification of **lysine**-71, whereas 7-chloro-4-nitro-2,1,3- benzoxadiazole (NBD-Cl) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) react with cysteines-199 and -343 to inactivate the enzyme. All three of these reagents have been postulated to modify residues at or near the active site of the catalytic subunit. ATP (2 mM) in the presence of excess Mg2+ (10 mM) protects the enzyme against inactivation by these reagents. AMP did not afford any protection, but adenosine slightly decreased the rate of inactivation. The specific effects of covalent modification of **lysine**-71 and cysteines-199 and -343 on nucleotide **binding** were characterized by fluorescence- polarization titrations with lin-benzoadenine nucleotides as fluorescent ligands. lin-Benzoadenosine is a competitive inhibitor of the catalytic subunit with respect to ATP with a Ki (38 microM) similar to the Ki for adenosine (35 microM). This value agrees well with the Kd (32 microM) for adenosine determined by fluorescence-polarization titrations. lin-Benzoadenosine 5'-diphosphate (lin-benzo-ADP) has been shown to be a competitive inhibitor with respect to ATP [Hartl, F. T.,

Roskoski, R., Jr., Rosendahl, M. S., & Leonard, N. J. (1983) Biochemistry 22, 2347], and lin-benzoadenosine 5'-triphosphate (lin-benzo-ATP) is a substrate for the phosphotransferase activity of the protein **kinase**.(ABSTRACT TRUNCATED AT 250 WORDS)

L24 ANSWER 8 OF 9 MEDLINE on STN
ACCESSION NUMBER: 84270751 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 6431300
TITLE: Direct evidence that oncogenic tyrosine **kinases** and cyclic AMP-dependent protein **kinase** have homologous ATP-**binding** sites.
AUTHOR: Kamps M P; Taylor S S; Sefton B M
CONTRACT NUMBER: CA-14195 (NCI)
CA-17289 (NCI)
GM-19301 (NIGMS)
SOURCE: Nature, (Aug 16-22 1984) Vol. 310, No. 5978, pp. 589-92.
Journal code: 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198409
ENTRY DATE: Entered STN: 20 Mar 1990
Last Updated on STN: 20 Apr 2002
Entered Medline: 17 Sep 1984

AB p60src, the transforming protein of Rous sarcoma virus (RSV), is a protein **kinase** that has a strict specificity for tyrosine. The phosphorylation of cellular proteins by p60src (reference 4) results in transformation. Recently, Barker and Dayhoff discovered that residues 259-485 of p60src have 22% sequence identity with residues 33-258 of the catalytic subunit of cyclic AMP-dependent protein **kinase**, an enzyme that has a specificity for serine. Because it was necessary to introduce eight gaps to align the two proteins, the question remained as to whether this apparent homology reflected a common evolutionary origin. We demonstrate here that the ATP analogue p-fluorosulphonylbenzoyl 5'-adenosine (**FSBA**) inactivates the tyrosine protein **kinase** activity of p60src by reacting with **lysine** 295. When aligned for maximum sequence identity, **lysine** 295 of p60src and the **lysine** in the catalytic subunit which also reacts specifically with **FSBA** are superimposed precisely. This functional homology is strong evidence that the protein **kinases**, irrespective of amino acid substrate specificity, comprise a single divergent gene family.

L24 ANSWER 9 OF 9 MEDLINE on STN
ACCESSION NUMBER: 81094098 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 6256383
TITLE: Affinity labeling of the ATP **binding** site of bovine lung cyclic GMP-dependent protein **kinase** with 5'-p-fluorosulfonylbenzoyladenosine.
AUTHOR: Hixson C S; Krebs E G
SOURCE: The Journal of biological chemistry, (1981 Feb 10) Vol. 256, No. 3, pp. 1122-7.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 198103
ENTRY DATE: Entered STN: 16 Mar 1990
Last Updated on STN: 20 Apr 2002
Entered Medline: 27 Mar 1981

AB Bovine lung cyclic GMP-dependent protein **kinase** was covalently labeled with the ATP analog, 5-p-fluorosulfonylbenzoyladenosine. The inactivation reaction was pseudo-first order. The rate of **kinase** sulfonation exhibited saturation kinetics indicative of a rapid reversible **binding** of the reagent prior to enzyme modification. The enzyme could be protected by MgATP, MgADP, and Mg-adenylylimidodiphosphate but not by a synthetic peptide substrate. Cyclic GMP when **bound** to the **kinase** did not influence the rate of labeling. The reagent demonstrated competitive inhibition with respect to MgATP; the Ki was found to be 0.82 mM. Magnesium and cobalt ions when included in the reaction mixture accelerated the inactivation rate up to severalfold. Addition of basic polypeptides such as mixed histone, protamine sulfate, or poly-L-**lysine** HBr also markedly accelerated the sulfonation rate. Inactivation of the **kinase** with 5- 'fluorosulfonyl[3H]benzoyladenosine resulted in a linear relationship between the residual phosphotransferase activity and the incorporation of up to 0.9 mol of reagent/mol of monomer.